

Immunohistochemical study of the gastrointestinal endocrine cells in the Mongolian Gerbils, *Meriones unguiculatus*

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Abstract : In order to study the regional distribution and relative frequency of the immunoreactive endocrine cells in the gastrointestinal tract of the Mongolian gerbil, *Meriones unguiculatus*, the gastrointestinal tract was divided into 9 portions (cardia, fundus, pylorus, duodenum, jejunum, ileum, cecum, colon and rectum) and immunostained by immunohistochemical (PAP) method using 8 types of specific antisera against cholecystokinin (CCK)-8, gastrin, secretin, pancreatic polypeptide (PP), somatostatin, serotonin, glucagon and insulin. CCK-8-, gastrin-, somatostatin- and serotonin-immunoreactive cells were demonstrated in this study. These immunoreactive cells were found in the gastric gland regions of the pylorus or between parietal and chief cells of the fundus with round to spherical shaped, and in the interepithelial regions of the intestinal tract with spherical to spindle shaped except for jejunum where some of immunoreactive cells were also observed in the intestinal glands with round to spherical shaped. CCK-8-immunoreactive cells were restricted to the pylorus and duodenum with numerous and a few frequency, respectively. Gastrin-immunoreactive cells were restricted to the pylorus with numerous frequency. Similar to those of gastrin-immunoreactive cells, somatostatin-immunoreactive cells were restricted to pylorus with moderate frequency. Serotonin-immunoreactive cells were detected throughout whole gastrointestinal tract except for cardia and cecum with moderate or numerous frequency. However, no secretin-, PP-, glucagon- and insulin-immunoreactive cells were observed in this study. From these results, the appearance type, regional distribution and relative frequency of immunoreactive endocrine cells in the gastrointestinal tract of the Mongolian gerbils were somewhat lowered or restricted compared to those of other mammals and these differences were might be caused by feeding habits and species specification.

Key words : Mongolian gerbil, gastrointestinal tract, endocrine cell, immunohistochemistry, immunoreactive cell.

Introduction

The Mongolian gerbil, *Meriones unguiculatus*, is a rodent of the family Cricetidae, although it has been included alternatively among the Muridae. The Mongolian gerbil is an active, nearly odorless, usually nonaggressive rodent distinguished by its monogamous mating behavior, water and temperature conservation mechanisms, spontaneous epileptiform seizures, relatively freedom from spontaneous disease, and several unique attributes of interest in research¹. Gastrointestinal endocrine cells dispersed in the epithelia and gastric glands of the alimentary

tract synthesize various kinds of gastrointestinal hormones and play an important role in the physiological functions of the alimentary tract². Until now, the investigation of gastrointestinal endocrine cells is considered to be an important part of a phylogenic study³. In addition, the regional distributions and relative frequencies of these endocrine cells were varied with animal species and feeding habits⁴. Although many studies have been elucidated the regional distribution and relative frequency of different endocrine cells in the alimentary tract of the various vertebrates including various species of rodents and in spite of the biological, physiological and

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anatomical difference from the other rodents, there was no reports about immunohistochemical studies on the gastrointestinal endocrine cells along whole gastrointestinal tract of the Mongolian gerbil except for following short pieces papers. Hawkins *et al*⁵ reported that chromogranin A-immunoreactive cells were observed in the stomach, small and large intestine of various laboratory animals including gerbil by immunohistochemical method and gastrin- and serotonin-like-immunoreactivity were detected in the duodenum of the gerbil⁶. In addition, Helmstaedter *et al*⁷ suggested that neurotensin- immunoreactive cells were existed in the small intestine of gerbils similar to those of other mammals. The purpose of the present study was to clarify the regional distribution and relative frequency of the endocrine cells in the gastrointestinal tract of the Mongolian gerbil, *Meriones unguiculatus* by immunohistochemical method using 8 types of specific antisera against cholecystokinin (CCK)-8, gastrin, secretin, pancreatic polypeptide (PP), somatostatin, serotonin, glucagon and insulin.

Materials and Methods

Five adult (40~50g body weight) Mongolian gerbils, *Meriones unguiculatus*, were acquired from Asan Institute for Life Science (Seoul, Korea) and were used in this study without sexual distinction. The animals were anesthetized with ethyl ether. After food restriction about 24 hours and phlebotomized, samples from the cardia, fundus, pylorus, duodenum, jejunum, ileum, colon, cecum and rectum were fixed in Bouin's solution. After paraffin embedding, 3-4 μ m serial sections were prepared. Representative sections of each tissue were stained with hematoxylin and eosin for light microscopic examination of the normal gastrointestinal architecture.

The each representative sections were deparaffinized, rehydrated and immunostained with the peroxidase anti-peroxidase (PAP) method⁸. Blocking of nonspecific reaction was performed with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsing in phosphate buffered saline (PBS; 0.01M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS buffer and finally the PAP complex was prepared. The peroxidase reaction was carried out in a solution 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H₂O₂ in Tris-HCl buffer (0.05M, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and

Table 1. Antisera used in this study

Antisera raised*	Code	Source	Dilution
CCK-8 ¹⁾	8643010	Immunonuclear Corp., Stillwater.	1 : 1,000
Gastrin	PUO190796	BioGenex Lab., San Ramon.	1 : 20
Secretin	BO67122A	BioGenex Lab., San Ramon.	1 : 20
PP ¹⁾	PUO660495	BioGenex Lab., San Ramon.	1 : 20
Somatostatin	PUO421295	BioGenex Lab., San Ramon.	1 : 20
Serotonin	BO68082C	BioGenex Lab., San Ramon	1 : 20
Glucagon	PUO390598	BioGenex Lab., San Ramon.	1 : 20
Insulin	PUO290395	BioGenex Lab., San Ramon.	1 : 20

*All antisera were raised in rabbits except for insulin, which was raised in rabbits.

¹⁾CCK-8: cholecystokinin-8, PP: pancreatic polypeptide

the immunoreactive cells were observed under light microscope.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger⁸, including the replacement of specific antiserum by the same antiserum, which had been preincubated with its corresponding antigen. The relative frequency of occurrence of each type of immunoreactive cell was placed into one of five categories according to their observed numbers as seen using light microscopy.

Results

In this study, four kinds of the immunoreactive endocrine cells were detected with the antisera against CCK-8, gastrin, somatostatin and serotonin in the gastrointestinal tract, but four kinds of immunoreactive endocrine cells were not demonstrated with the antisera against secretin, PP, glucagon and insulin. According to the location of the gastrointestinal tract, different regional distribution and relative frequencies of these immunoreactive cells were observed. These differences are shown in Table 2. Spherical to spindle and/or oval to round-shaped immunoreactive cells were located in the gastric glands of stomach regions, in the basal portion of the epithelium and/or in the intestinal gland (in a case

Table 2. Regional distributions and relative frequencies of the endocrine cells in the gastrointestinal tract of the Mongolian gerbil, *Meriones unguiculatus*

	Cardia	Fundus	Pylorus	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum
CCK-8 ¹⁾	-	-	+++	+	-	-	-	-	-
Gastrin	-	-	+++	-	-	-	-	-	-
Secretin	-	-	-	-	-	-	-	-	-
PP ¹⁾	-	-	-	-	-	-	-	-	-
Som ¹⁾	-	-	++	-	-	-	-	-	-
Serotonin	-	++	+++	++	++	++	-	+++	++
Glucagon	-	-	-	-	-	-	-	-	-
Insulin	-	-	-	-	-	-	-	-	-

¹⁾CCK-8: cholecystokinin-8; PP: pancreatic polypeptide; Som: somatostatin.

*Relative frequencies; +++: numerous, ++: moderate, +: a few, ±: rare, -: not detected

Fig 1. CCK-8-immunoreactive cells in the pylorus (a) and duodenum (b) of the Mongolian gerbil.

a: × 150; b: × 600. PAP method.

Fig 2. Gastrin-immunoreactive cells in the pylorus of the Mongolian gerbil, × 150.

of the jejunum) of intestinal tract with variable frequencies.

CCK-8-immunoreactive cells were restricted to the

Fig 3. Somatostatin-immunoreactive cells in the pylorus of the Mongolian gerbil, × 300.

pylorus and duodenum with numerous and a few frequency, respectively (Table 2). They were situated in the basal portion of pyloric mucosa especially intergastric gland regions with round to spherical shaped (Fig 1a) and were also detected in the interepithelial regions of duodenal mucosa (Fig 1b).

Gastrin-immunoreactive cells were restricted to the pylorus with numerous frequency (Table 2). These immunoreactive cells were located in the basal portion of pyloric mucosa especially intergastric gland regions with round to spherical shaped (Fig 2).

Somatostatin-immunoreactive cells were restricted to the pylorus with moderate frequency (Table 2) and situated in basal portion of pyloric mucosa especially intergastric gland regions with round to spherical shaped (Fig 3).

Serotonin-immunoreactive cells were demonstrated whole gastrointestinal tract except for the cardia and

Fig 4. Serotonin-immunoreactive cells in the gastrointestinal tract of the Mongolian gerbil

a. Fundus, b, c. Pylorus, d. Duodenum, e. Jejunum, f. Ileum, g-i. Colon, j-l. Rectum (a, c, f, h, I, k, l: $\times 600$; d, e: $\times 300$; b, g, j: $\times 150$, PAP method.)

cecum with various relative frequencies (Table 2). Moderated frequency of serotonin-immunoreactive cells was dispersed in the body of the fundus with round to spherical shaped (Fig 4a). They were situated in basal portion of pyloric mucosa especially intergastric gland regions with numerous frequency and they were round to spherical shaped (Fig 4b,c). In the duodenum, moderated frequency of serotonin-immunoreactive cells were dispersed in the interepithelial regions of the mucosa with spherical to spindle shaped (Fig 4d). Round to

spherical shaped cells were located in the intestinal glands of the jejunum with moderate frequency and a few cells were demonstrated in the interepithelial regions with spherical to spindle shaped (Fig 4e). In the ileum, moderate frequency of serotonin-immunoreactive cells was observed in the interepithelial regions with spherical shaped (Fig 4f). In the colon, they were situated in the interepithelial regions with numerous frequency and they were spherical to spindle shaped (Fig 4g,h). In addition, some of these immunoreactive cells were grouped in

these sites (Fig 4g,i). Moderate frequency of serotonin-immunoreactive cells were located in the interepithelial regions of the rectum with spindle shaped (Fig 4j,k) and some of these cells were situated in the basal portion of the epithelium with spherical shaped (Fig 4j,l).

Discussion

The endocrine cells in the alimentary tracts appeared remarkably different in the regional distribution, relative frequency and cell types with animal species and each regional part of the gastrointestinal tract^{9,10}. Different from those of other rodents, Mongolian gerbils of both sexes have a distinct midventral abdominal pad composed of large sebaceous glands under control of gonadal hormones¹¹, and Mongolian gerbil has unique feeding habits¹. In addition, male gerbils have higher packed red-cell volumes (PCV), hemoglobin levels, total leukocyte counts, and circulating lymphocyte counts than do females and some erythrocytes of both sexes have a prominent polychromasia and basophilic stippling¹. In spite of the biological, physiological and anatomical difference from the other rodents, there was no reports about immunohistochemical studies on the gastrointestinal endocrine cells along whole gastrointestinal tract of the Mongolian gerbil except for some of short papers.

It is generally accepted that gastrin and CCK-8 originated from same ancestor and in the human duodenum a large fraction of these cells, besides reacting with non-C terminal CCK antibodies and C-terminal gastrin/CCK antibodies, also show immunoreactivity with C-terminal gastrin-34 antibodies, colocalized with CCK in a variable portion of secretory granules¹². Gastrin secreted by intestinal G cell, was promoted the gastric acid secretion and CCK secreted by intestinal I cell was stimulated the pancreatic enzyme secretion. In present study, gastrin-immunoreactive cells were restricted to the pylorus and CCK-8-immunoreactive cells were found in the pylorus and duodenum. Generally, it is well known that gastrin- and CCK-immunoreactive cells were located in the gastric mucosa and whole small intestinal tract in mammals¹³⁻¹⁵. However, Lee *et al*¹⁶ reported that different from other mammalian species, gastrin/CCK-immunoreactive cells were abundant in the pyloric gland region but scarce in the duodenum and no cells were found in the other gastrointestinal regions of the Korean tree squirrel. Somewhat different from other mammalian species¹³⁻¹⁵, these immunoreactive cells were showed more

restricted distributional patterns in the present study, but these results were well corresponded to the reports in the Korean tree squirrel¹⁶.

In all mammalian animals so far investigated secretin-immunoreactive cells proved to be exclusive to the small intestine, usually with preference for the duodenum and upper jejunum^{12,13,17} but Kitamura *et al*¹⁵ reported that these immunoreactive cells were detected in the small and large intestinal tract of the musk shrew. However, no secretin-immunoreactive cells were detected in this study. Since PP was isolated from insulin extraction of pancreas in 1961, the regional distribution of PP-immunoreactive cells in the mammalian species was relatively well known but species-dependend differences were existed among the mammals^{13,14,16,17}. However, quite differed from previous reports, no PP-immunoreactive cells were demonstrated in this study^{13,14,16,17}.

Somatostatin, consisted of 14 amino acids, was isolated from hypothalamus of sheep for the first time and it could be divided into straight form and cyclic form¹⁸. This substance inhibits the secretion of the other neuroendocrine hormones¹⁹. It is known that somatostatin-immunoreactive cells show the widest distribution in the whole gastrointestinal tract of all vertebrate species investigated, including the primitive agnathans with serotonin-immunoreactive cells²⁰. However, these immunoreactive cells were restricted to the abomasums and small intestine of the Philippine carabao¹⁷ and restricted to the second stomach of the striped dolphin²¹. Different from most of mammals²⁰ and in the Philippine carabao¹⁷ but similar to those of the striped dolphin²¹, somatostatin-immunoreactive cells were restricted to the pylorus in this study.

Serotonin was consisted of monoamines and widely distributed in nervous system and GEP endocrine cells²². Main functions of serotonin were inhibition of gastric acid secretion and contraction of smooth muscle in the gastrointestinal tract²³. El-Salhy *et al*²² (1985) reported that serotonin-immunoreactive cells were detected throughout the gastrointestinal tract of all species and established in the GIT at the early stage of vertebrate evolution. In addition, these immunoreactive cells were detected in the whole alimentary tract including esophagus of low vertebrate^{24,25}. Serotonin-immunoreactive cells were detected in the whole gastrointestinal tract of the common tree shrew¹³, Philippine carabao¹⁷, lesser mouse deer¹⁴ and rat²⁶, but Domeneghini *et al*²¹ reported that these immunoreactive cells were not detected in the striped dolphin. In the present study, serotonin-immunoreactive

cells were demonstrated in the whole gastrointestinal tract except for cardia and cecum. These results considered as similar to most of other mammals^{13,14,17,26}.

Glucagon is synthesized in the A cells of the pancreas and regulated serum glucose levels. These immunoreactive cells have been demonstrated in various mammals. They were demonstrated in the whole gastrointestinal tract of the common tree shrew¹³, lesser mouse deer¹⁴ and musk shrew¹⁵ but Baltazar *et al*¹⁷ persisted that these immunoreactive cells were only detected in the intestinal tract of the Philippine carabao and Lee *et al*¹⁶ reported that they were restricted to the cardia and fundus of the Korean tree squirrel. According to the above works, it is suggested that the regional distribution of glucagon-immunoreactive cells were varied with mammalian species. However, no glucagon-immunoreactive cells were observed in the Mongolian gerbils.

Insulin is synthesized in the B cells of the pancreatic islets and regulated the serum glucose levels²⁷. It is generally accepted that insulin containing cells migrated from mucosal epithelium of the intestine early in vertebrate evolution to the acinar pancreas and no insulin-immunoreactive cells were detected in the whole gastrointestinal tract except for turtles, *Chrysemys dorsalis* and *Phrynosoma marmoratus* where these immunoreactive cells were detected in the gastric antrum and gut with spherical to spindle shape²⁸. In addition, Reinecke *et al*²⁹ reported that transient insulin-immunoreactive endocrine cells were detected from 5 to 10 days after hatching in the stomach and intestine of turbot, *Scophthalmus maximus*. Anyway, similar to most of other mammals, no insulin-immunoreactive cells were detected in this study.

In conclusion, the appearance type, regional distribution and relative frequency of immunoreactive endocrine cells in the gastrointestinal tract of the Mongolian gerbils were somewhat lowered or restricted compared to those of other mammals and these differences were might be caused by feeding habits and species specification.

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몽골리안 저빌(*Meriones unguiculatus*)의 위장관 내분비 세포에 대한 면역조직화학적 연구

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국문초록 : 몽골리안 저빌(*Meriones unguiculatus*)의 위장관을 분문부, 기저부, 유문부, 십이지장, 공장, 회장, 맹장, 결장 및 직장의 9부분으로 구분하고 이들 부위에서 cholecystokinin(CCK)-8, gastrin, secretin, pancreatic polypeptide(PP), somatostatin, serotonin, glucagon 및 insulin 등 8종의 항혈청에 대한 내분비 세포의 부위별 분포 및 출현빈도를 면역조직화학적으로 관찰하였던 바, CCK-8, gastrin, somatostatin 및 serotonin 면역반응세포들이 관찰되었다. 이들 면역반응세포들은 위 부위에서는 원형에서 타원형으로 주로 위샘(gastric gland) 또는 주세포와 벽세포 사이에서 관찰되었고, 장 부위에서는 원형 또는 타원형의 면역반응세포들이 공장의 장샘(intestinal gland)에서 관찰되었으며, 타원형에서 방추형의 세포들이 장상피세포 사이에서 관찰되었다. CCK-8 면역반응세포들은 유문부와 십이지장에 국한되어 다수 또는 소수 관찰되었으며, gastrin 면역반응세포들은 유문부에 국한되어 다수 관찰되었다. Somatostatin 면역반응세포들 역시 gastrin 면역반응세포들과 유사하게 유문부에 국한되어 중등도의 출현빈도를 나타내었고, serotonin 면역반응세포들은 분문부와 맹장을 제외한 위장관 전부위에 걸쳐 중등도 또는 다수 관찰되었으나, secretin, PP, glucagon 및 insulin 면역반응세포들은 관찰되지 않았다. 이상에서 몽골리안 저빌의 위장관에 존재하는 내분비 세포들의 종류 및 출현빈도는 다른 설치류에 비해 매우 적거나 낮은 것으로 관찰되었으며, 이들 내분비 세포들의 출현부위 역시 다른 설치류에서와는 달리 일부 부분에 국한되는 것으로 관찰되었다.

Key words : Mongolian gerbil, gastrointestinal tract, endocrine cell, immunohistochemistry, immunoreactive cell